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Spectroscopic analyses of chemical adaptation processes within microalgal biomass in response to changing environments

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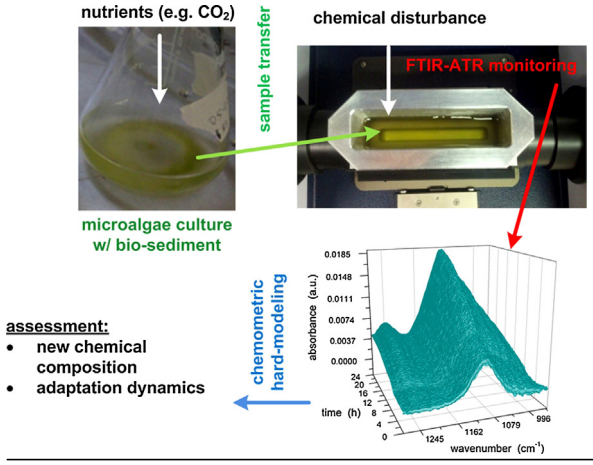
HIGHLIGHTS

- Microalgae transform large quantities of inorganics into biomass.
- Microalgae interact with their growing environment and adapt their chemical composition.
- Sequestration capabilities are dependent on cells' chemical environments.
- We develop a chemometric hard-modeling to describe these chemical adaptation dynamics.
- This methodology will enable studies of microalgal compound sequestration.

GRAPHICAL ABSTRACT



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ABSTRACT

Via photosynthesis, marine phytoplankton transforms large quantities of inorganic compounds into biomass. This has considerable environmental impacts as microalgae contribute for instance to counter-balancing anthropogenic releases of the greenhouse gas CO_2. On the other hand, high concentrations of nitrogen compounds in an ecosystem can lead to harmful algae blooms. In previous investigations it was found that the chemical composition of microalgal biomass is strongly dependent on the nutrient availability. Therefore, it is expected that algae's sequestration capabilities and productivity are also determined by the cells' chemical environments. For investigating this hypothesis, novel analytical methodologies are required which are capable of monitoring live cells exposed to chemically shifting environments followed by chemometric modeling of their chemical adaptation dynamics.

FTIR-ATR experiments have been developed for acquiring spectroscopic time series of live *Dunaliella parva* cultures adapting to different nutrient situations. Comparing experimental data from acclimated cultures to those exposed to a chemically shifted nutrient situation reveals insights in which analyte groups participate in modifications of microalgal biomass and on what time scales. For a chemometric description of these processes, a data model has been deduced which explains the chemical adaptation dynamics explicitly rather than empirically. First results show that this approach is feasible and derives information about the chemical biomass adaptations. Future investigations will utilize these

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instrumental and chemometric methodologies for quantitative investigations of the relation between chemical environments and microalgal sequestration capabilities.

1. Introduction

With an increasing industrialization, the production of anthropogenic CO_2 is rising [1] and the fate of this greenhouse gas has become a serious concern [2,3]. On the other hand, one major sink of atmospheric CO_2 is photosynthesis and since marine phytoplankton is responsible for ~50% of the global primary production [4–10], it considerably contributes to counter-balancing anthropogenic CO_2 releases [11–13]. In previous studies, the amount of produced microalgal biomass [14–16] and its chemical composition [17–20] have been linked to nutrient availability (C,[1] N, P, Fe, and S) [21]. Apparently, the cells' chemical nutrient utilization shifts when their chemical environment changes. For an accurate qualitative and quantitative assessment of a microalgal community's sequestration of CO_2 and other inorganic nutrients into biomass, an understanding of phytoplankton's acclimatization mechanisms is crucial. Gathering such information on a culture-level can then contribute to a more accurate evaluation of the oceans' carbon storage capacities which show first signs of saturation [22].

To investigate environmental impacts on phytoplankton biomass, HPLC [23–25] and FTIR spectroscopy [17,26–31], have been utilized; the latter technique in particular has gained popularity due to its sensitivity and selectivity for numerous, biologically relevant compounds [32]. The fact that nutrient levels and the presence of nutrient competitors are reflected in the biomass' IR-spectroscopic signatures has been utilized in Refs. [18,19], for indirect analyses of growing environments. Another study [33] developed a novel chemometric methodology for FTIR-based quantitation of selected compounds within phytoplankton biomass. In these experiments, however, the biomass had to be fixed and thus only facilitated discontinuous analyses of the biomass' chemical composition. Moreover, the algae's chemical composition may be falsified during the biomass fixation as in particular volatile compounds may be lost during the drying step. Thus, for monitoring phytoplankton's adaptation to a chemical shift in its growing environment, a novel approach is required that facilitates studying live cells. The required aqueous environment in turn hampers the use of FTIR transmission spectroscopy. In order to utilize the proven sensitivity and selectivity of FTIR-spectroscopy, this study employed FTIR in attenuated total reflection (ATR) mode.

2. Experimental

Cultures of the sea water species *Dunaliella parva* (The Culture Collection of Algae at the University of Texas at Austin) were grown in 'semi-continuous mode' [34] for eight days while being exposed to continuous illumination and 20 °C [14,18]. The cells were cultured in standard 'Enriched Seawater, Artificial Water' (ESAW) medium [34,35], which contains natural nutrient concentration levels namely 2071 μM HCO_3^- and 549 μM NO_3^-. These acclimated cultures were then transferred into a horizontal ATR accessory (PIKE Technologies) with a custom made ~100 mL liquid cell placed on top of it. For the following 24 h, FTIR-ATR spectra (4 cm^{-1} resolution, 128 co-added scans) were recorded in 4 min intervals with a Bruker Vertex 70 equipped with a DTGS detector. For subsequent chemometric studies, the wavenumber range 1350–950 cm^{-1} had been chosen since many biologically relevant compounds namely sugars feature a distinct IR signature in this region [32]. During this 24 h period, the microalgae cells slowly sink to the bottom (Fig. 1(a)) of the horizontal ATR cuvette where they are then probed by the mid-IR evanescent field. During this sinking process, the cells adapt to their chemical (nutrient) environment. Therefore, two processes determine the time dependent spectroscopic signatures: the accumulation of biomass within the reach of the evanescent field and changes in the cells' chemical composition. In order separate both influences on the spectra, two types of experiments have been conducted: (i) phytoplankton cultures were spectroscopically monitored without modification of their nutrient situation which thereby established a benchmark. The second type of experiment considered (ii-a) dilution of the nitrate concentration from 549 μM to 419 μM which has been achieved by adding 25 mL of 160 μM nitrate to 50 mL of standard ESAW (549 μM NO_3^-) in which the cells are contained. The other nutrients in the 25 mL top-off were kept at the standard ESAW level and thus no dilution was introduced. (ii-b) Within other cell cultures, the nitrate concentration had been increased from 549 μM to 793 μM, 856 μM, and 916 μM, respectively, by adding 25 mL of a higher concentrated ESAW to the 50 mL standard ESAW. All cultures were prepared and analyzed in triple replicates.

Fig. 1(b) depicts a time series of FTIR-ATR spectra acquired from a microalgae culture after spiking it to an elevated NO_3^- concentration. The increase of absorbance over time is clearly visible and a closer look reveals that in the beginning of the time series the absorption somewhat decreased. These changes in absorbances are hypothesized to be due to bio-sediment build-up and modification of the biomass' chemical composition.

3. Theory – modeling biomass' physical and chemical dynamics

In order to explain such spectroscopic time series, a model function $Y(\tilde{\nu}, t) = A(t) \times \varepsilon(\tilde{\nu}, t)$ for the measured data has been deduced which comprises of two factors, i.e., a time-dependent term $A(t)$ which describes the accumulation of biomass within the reach of the evanescent field and a term, $\varepsilon(\tilde{\nu}, t)$, which describes wavenumber and time-dependent spectroscopic features. While only $\varepsilon(\tilde{\nu}, t)$ is of immediate chemical and biological interest, the introduction of $A(t)$ is required to properly explain the measured spectra. This model function $Y(\tilde{\nu}, t)$ is loosely related to Beer's Law by interpreting $A(t)$ as the biomass' "concentration" which increases over time and $\varepsilon(\tilde{\nu}, t)$ as wavenumber- and time-dependent "molar absorptivity". Furthermore, as shown below, $Y(\tilde{\nu}, t)$ contains numerous model parameters θ which reflect properties of a specific sample and will be extracted from experimental data via nonlinear least-squares regression. The long-term goal is to relate the values of the θs to ambient chemical parameters and thus to explicitly link the chemical environment to the microalgae's responses. Since $Y(\tilde{\nu}, t)$ is derived from theoretical considerations rather than in an empirically fashion, these θ will carry interpretable spectrochemical and/or physical meanings based on which the key players of phytoplankton's adaptation

[1] Note: sequestering atmospheric CO_2 by phytoplankton in aqueous media occurs mostly by uptake of HCO_3^- [9] which is produced via: $CO_{2(g)} \leftrightarrow CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$.